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REVIEW

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Senescent cells in the development of cardiometabolic disease

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Purpose of review
Senescent cells have recently been identified as key players in the development of metabolic dysfunction. In this review, we will highlight recent developments in this field and discuss the concept of targeting these cells to prevent or treat cardiometabolic diseases.

Recent findings
Evidence is accumulating that cellular senescence contributes to adipose tissue dysfunction, presumably through induction of low-grade inflammation and inhibition of adipogenic differentiation leading to insulin resistance and dyslipidaemia. Senescent cells modulate their surroundings through their bioactive secretome and only a relatively small number of senescent cells is sufficient to cause persistent physical dysfunction even in young mice. Proof-of-principle studies showed that selective elimination of senescent cells can prevent or delay the development of cardiometabolic diseases in mice.

Summary
The metabolic consequences of senescent cell accumulation in various tissues are now unravelling and point to new therapeutic opportunities for the treatment of cardiometabolic diseases.

Keywords
cardiometabolic disease, cellular senescence, insulin resistance, metabolic syndrome, senolytics

INTRODUCTION

Cellular or replicative senescence is a protective response against endogenous and exogenous stressors in which cells permanently arrest their cell-cycle and undergo phenotypic alterations. The term senescence was initially introduced in 1961 by Hayflick and Moorhead [1] who observed that fibroblasts in culture were only able to divide a limited number of times before entering a state of permanent growth arrest. Compelling in-vivo evidence for this concept had long been lacking, until the discovery that cellular senescence can act as a potent cancer defence mechanism that prevents the proliferation of preneoplastic cells [2]. Furthermore, cellular senescence is a fundamental player in a range of physiological and pathophysiological processes, including wound healing, embryogenesis, ageing and the development of age-related diseases [3–10]. A variety of stimuli can induce cellular senescence, including telomere shortening (replicative senescence), oncogenic activity (oncogene-induced senescence) and stressors such as DNA damage, oxidative stress, inflammatory mediators and metabolites (stress-induced premature senescence) that induce growth arrest via activation of the p53-p21 and $p16^{Ink4a}$/Rb tumour suppressor pathways [11–13]. Senescent cells display several hallmarks, including upregulation of the cyclin-dependent kinase inhibitor $p16^{Ink4a}$, increased senescence-associated lysosomal β-galactosidase activity (SA-β-gal) and a characteristic secretome consisting of pro-inflammatory cytokines, chemokines, growth

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KEY POINTS

- Senescent cells contribute to adipose tissue dysfunction leading to systemic metabolic alterations.
- Adipose dysfunction leads to accelerated accumulation of senescent cells in other tissues.
- Senescent cells contribute to the development and progression of NAFLD.
- Selective elimination of senescent cells in an atherosclerotic-prone mouse model results in decreased lesion formation and increased plaque stability.

factors and proteases, the so-called senescence-associated secretory phenotype (SASP) [14–19]. The SASP is enriched for components that can attract immune cells and are thought to mediate natural elimination of senescent cells. However, during biological ageing, senescent cells accumulate within tissues, presumably due to increased cellular stress with ageing and deterioration of the immune system [20–26]. Remarkably, only a relatively small number of senescent cells seemingly is sufficient to drive tissue dysfunction throughout the body. Persistent SASP secretion may trigger senescence in distantly located cells [8,27,28,29▪▪], thereby perhaps promoting chronic tissue inflammation and degeneration. In this review, we delineate what is currently known about the role of senescent cells in metabolic and cardiovascular disorders.

CELLULAR SENESCENCE AS A CAUSE OF METABOLIC DYSFUNCTION

Ageing is the major risk factor for the development of multiple chronic diseases and decline in physical functions. The first evidence that senescent cells are causally involved in ageing came from studies by Baker *et al.* [30,31], who examined the budding uninhibited by benomyl related-1 hypomorphic mice (BubR1$^{H/H}$), which show accelerated ageing due to low levels of the core mitotic checkpoint protein BubR1 (Table 1) [30,31]. Inducible elimination of p16^{Ink4a}-positive cells from BubR1$^{H/H}$ mice delayed the onset of age-related diseases such as sarcopenia, cataract and lipodystrophy (Table 1). Later studies in naturally aged mice confirmed this relationship and demonstrated that elimination of senescent cells extended life span [8]. Although senescent cells accumulate with ageing in multiple tissues, recent studies have raised the possibility that

Table 1. Clarification of the mouse models used to study the relationship between senescence and cardiometabolic diseases

Mouse model	Description	Major findings	References
BubR1 hypomorphic mouse (BubR1$^{H/H}$)	BubR1 is a core protein of the spindle assembly checkpoint, a safeguard that ensures correct chromosome segregation. BubR1 hypomorphic mice produce 10% of the BubR1 protein.	BubR1$^{H/H}$ is a model of accelerated ageing, as mice show markedly shortened lifespan and display several age-related diseases, including sarcopenia, cataracts, fat loss, arterial wall stiffening and impaired wound healing. BubR1$^{H/H}$ mice accumulate p16^{Ink4a}-positive cells in several tissues, including adipose tissue, skeletal muscle and eye.	[30,31]
INK-ATTAC naturally aged mouse	The *INK-ATTAC* transgene allows for selective elimination of p16^{Ink4a}-positive cells upon administration of the synthetic drug AP20187, which induces dimerization of a membrane-bound myristoylated FK506-binding-protein-caspase 8 (FKBP-Casp8) fusion protein expressed specifically in senescent cells via the p16^{Ink4a} promoter. Furthermore, an internal ribosome entry site (IRES) followed by an open reading frame (ORF) coding for enhanced green fluorescence protein (EGFP), which allows detection and collection of p16^{Ink4a}-positive senescent cells is incorporated in the construct.	Clearance of p16^{Ink4a}-positive cells resulted in increased lifespan in male and female mice, delayed tumorigenesis and attenuated age-related diseases, including lipodystrophy, kidney dysfunction and cardiac dysfunction. Mechanistically, elimination of p16^{Ink4a}-positive cells enhanced adipogenic transcription factors, reduced circulating levels of activin A and reduced fat accumulation in the liver of aged mice.	[8,32,33▪▪]
INK-ATTAC BubR1$^{H/H}$ mouse	Incorporation of the *INK-ATTAC* transgene in the progeroid BubR1$^{H/H}$ mouse.	Removal of p16^{Ink4a}-positive cells in a model of accelerated ageing resulted in delayed onset of age-associated features, including sarcopenia, cataracts and lipodystrophy.	[30]

cellular senescence in adipose tissue may promote age-related diseases and frailty [34].

Senescence as inducer of adipose tissue dysfunction

Adipose tissue is an active and dynamic endocrine organ that apart from its primary function to store energy in the form of fats also regulates systemic metabolism in response to nutrient intake, lifestyle and environmental changes [35]. With ageing, the distribution and function of adipose tissue changes significantly [35]. Old age is associated with a marked reduction in subcutaneous white adipose tissue (sWAT) and brown adipose tissue (BAT) and increased presence of visceral WAT (vWAT), accompanied by diminished lipid handling, altered secretion of adipokines, low-grade inflammation, defective thermogenesis and de-novo adipogenesis, which combined contributes to the development of insulin resistance and dyslipidaemia [35–39]. Senescent preadipocytes were shown to accumulate in BubR1 progeroid mice wherein they were first shown to cause lipodystrophy [31,40], a finding that was later confirmed in naturally aged mice [8]. Removal of senescent cells in mice resulted in a reduction of the pro-inflammatory SASP factor interleukin 6 (IL-6). Senescent cell accumulation can be accelerated in mice by excessive calorie intake and genomic instability [28,41]. In humans, obesity and diabetes is associated with senescent cell accumulation in adipose tissue, which correlated with adipose tissue dysfunction [28,35,41].

Senescent preadipocytes that cease to divide may limit the ability of the adipose tissue to expand, a process essential for storage of excess nutrients and to maintain metabolic health during obesity. During adipogenesis, preadipocytes can differentiate into insulin-responsive white adipocytes that store fats or into beige adipocytes that control thermogenesis by converting glucose and fats into heat [42,43]. The potential to form white and beige adipocytes declines with age [44–47]. Senescent adipocyte progenitors from fat pads of elderly human donors displayed markedly reduced levels of adipogenic transcription factors [peroxisome proliferator activated receptor gamma 2 (PPARγ2) and CCAAT/enhancer-binding protein alpha (C/EBPα)] and mature adipocyte markers (leptin, adiponectin, fatty acid-binding protein 4) as well as reduced adipogenic capacity of preadipocytes in culture [32,48]. Activin A, a member of the transforming growth factor beta superfamily and a critical inhibitor of proliferation and differentiation of preadipocytes, was identified as an important component of the senescent cell secretome and impaired adipogenesis in neighbouring, nonsenescent progenitors [32,49].

A study by Berry *et al.* [50▪] targeting the main regulators of cellular senescence p21 and $p16^{Ink4a}$ revealed that upregulation of *p21* disrupted the potential of beige progenitors to differentiate into cold-induced beige adipocytes in mice. Both deletion and pharmacological inhibition of the p38/MAPK-$p16^{Ink4a}$ pathway were able to reverse this phenotype and resulted in improved glucose sensitivity [50▪]. Similarly, adipocyte-specific deletion of p53 or inhibition of p53 using pifithrin-α resulted in enhanced beige adipocyte formation upon cold exposure, increased energy expenditure and improved glucose clearance. Mechanistically, increased expression of p53 in aged adipose tissue appears to prevent adipocyte beiging through stimulation of mitophagy and prevention of increase in mitochondrial mass necessary for white-to-beige adipocyte conversion [51]. AP20187 treatment of naturally aged mice carrying the *INK-ATTAC* transgene, which allows for selective elimination of $p16^{Ink4a}$-positive cells upon administration of AP20187 (Table 1), resulted in reduced circulating levels of activin A, enhanced expression of adipogenic transcription factors and reduced fat loss, although it should be noted that clearance of senescent cells was not demonstrated [32]. Nevertheless, these data are consistent with the idea that cellular senescence impairs adipogenesis, which can result in fatty acid spill over and ectopic lipid deposition in other organs promoting insulin resistance, nonalcoholic fatty liver disease (NAFLD) and atherosclerosis [52].

In addition to adipocytes, endothelial cells lining the microvasculature of the adipose tissue determine adipose tissue mass. Previous work by Kanda *et al.* [53] demonstrated a critical role for endothelial PPARγ in adipose tissue expansion in response to a high fat diet. Deletion of PPARγ from endothelial cells resulted in reduced adipose tissue mass and adipocyte size [53]. Accumulating evidence suggests that cellular senescence can affect the adipose tissue endothelial cells thereby impairing fatty acid handling and enhancing immune cell infiltration [54]. Interestingly, visceral adipose tissue depots isolated from obese individuals showed enhanced expression of pro-inflammatory mediators and senescence markers and reduced expression of metabolism-related genes as compared to subcutaneous adipose tissue [54,55]. Recently, Briot *et al.* [54] demonstrated that activation of PPARγ using its agonist rosiglitazone stimulated fatty acid uptake through expression of fatty acid transporters *FATP1*, *FATP4* and *CD36* in endothelial cells isolated from human adipose tissue. Remarkably, after induction of replicative senescence, activation of PPARγ by

rosiglitazone promoted expression of pro-inflammatory mediators instead of fatty acid transporters [54]. The molecular events behind this surprising shift in PPARγ transcriptional activity remain to be elucidated. PPARγ was recently identified to act upstream of methyltransferase SETD8, which catalyses methylation of histone H4 at lysine 20 (H4K20me) and thereby silences expression of *p16*^{Ink4a} and *p21* [56,57]. It remains to be investigated whether this mechanism also plays a role in endothelial cells.

Senescent cells that accumulate in adipose tissue during ageing, obesity and diabetes can disrupt the adipose tissue microenvironment via secretion of SASP components, thereby promoting adipose tissue inflammation and insulin resistance [35]. Recent studies highlight the murine double minute 2 (MDM2)-p53 axis as essential player in senescence-associated adipocyte dysfunction. Adipocyte-specific ablation of p53 in a mouse model of type 2 diabetes mellitus (T2DM) resulted in reduced senescent cell accumulation, reduced adipose tissue inflammation and improved insulin resistance. Conversely, p53 overexpression induced adipocyte senescence together with a pro-inflammatory environment causing impaired insulin sensitivity [41]. Ageing reduces the expression of *Mdm2*, an upstream inhibitor of p53 in WAT and BAT [58▪]. Adipocyte-specific deletion of *Mdm2* resulted in age-dependent lipodystrophy caused by p53-dependent induction of apoptosis and senescence, which was associated with development of T2DM, NAFLD and hyperlipidaemia [58▪]. Inhibition of p53 attenuated senescence in adipose tissue, improved adipose function together with insulin sensitivity and glucose tolerance in a mouse model with elevated DNA damage due to *Polh* gene ablation [59]. Suppression of the janus kinase (JAK)-signal transducer and activator of transcription pathway, known for its role in regulating cytokine production, in aged mice reduced both adipose tissue and systemic inflammation, preserved fat mass, increased insulin sensitivity and reduced lipotoxicity [32]. Although these studies indicate that reduction of adipose inflammation resolves systemic dysfunction, they do not provide direct evidence for involvement of senescent cells in adipose tissue, as p53 or JAK inhibitors have pleiotropic effects on multiple tissues.

Very recently, adverse systemic effects of senescent adipose tissue cells were revealed via transplantation experiments of senescent adipocyte progenitors into fat tissue of healthy young mice. Senescent cells conferred senescence induction of host cells, not only locally but also in other tissues such as skeletal muscle, resulting in long-lasting physical dysfunction [29▪▪]. Transplantation of small numbers of senescent cells into aged or metabolically stressed mice resulted in more severe systemic dysfunction, accompanied with reduced survival of older recipients. Interestingly, replacement of senescent adipose tissue from adipocyte-specific *Mdm2* knockout mice by healthy adipose tissue from wild-type mice largely reversed glucose intolerance, insulin resistance and hyperlipidaemia and partially reduced senescence markers in liver and skeletal muscle [58▪]. Collectively, these findings indicate adverse effects of senescent cells on adipose tissue function via induction of low-grade inflammation and insulin resistance, which could be accompanied by effects on distant tissues important for metabolic control possibly through secretion of SASP factors (Fig. 1).

THE ROLE OF CELLULAR SENESCENCE IN METABOLIC SYNDROME RELATED COMPLICATIONS

Accumulation of senescent cells in adipose tissue seems to play an important causal role in accelerated development of metabolic syndrome with age. Insulin resistance and dyslipidaemia are important inducers of metabolic diseases, such as T2DM, NAFLD and cardiovascular diseases. Here, we will discuss the latest research regarding the role of senescence in the pathophysiology of NAFLD and atherosclerosis.

Role of senescence in nonalcoholic fatty liver disease

Metabolic syndrome is a major risk factor for the development and progression of NAFLD, the most common liver disease worldwide and one of the most serious diseases associated with obesity with an estimated worldwide prevalence of 25% [60]. NAFLD is characterized by accumulation of excess fat within hepatocytes (steatosis). Although in itself relatively benign, it progresses in approximately 25% of all patients into nonalcoholic steatohepatitis (NASH), which can eventually develop into more serious conditions such as fibrosis, cirrhosis and hepatocellular carcinoma (HCC) [61]. Senescent cell accumulation has been reported in human livers, which correlated with T2DM, hepatic steatosis progression and fibrosis stage [22,62–64]. Excessive calorie intake in mice resulted in upregulation of senescence markers in hepatocytes, which was closely correlated with lipid deposition in the liver and was ameliorated by both dietary restriction and exercise [28,33▪▪,41,65,66]. A disturbed metabolic homeostasis can also trigger senescent cell accumulation associated with liver steatosis as seen in

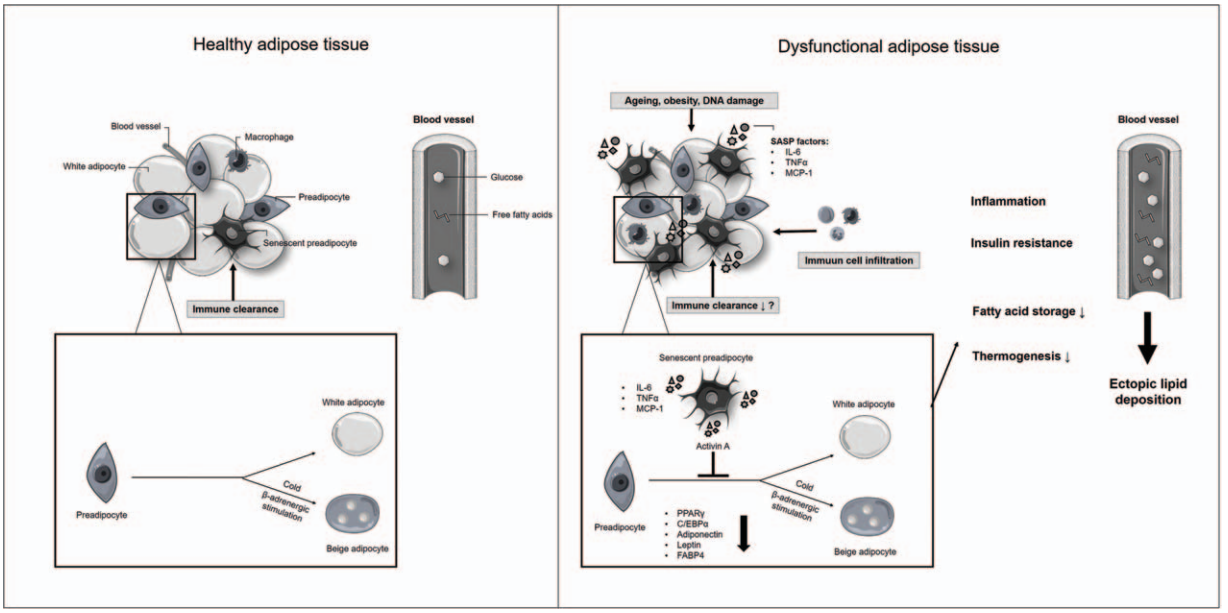

FIGURE 1. Potential mechanisms by which senescent cells contribute to adipose tissue dysfunction. Healthy adipose tissue is able to adapt to nutrient availability and environmental changes through adipogenesis providing metabolic flexibility. Senescent cell accumulation leads to increased secretion of SASP factors which can attract immune cells leading to low grade inflammation, insulin resistance and decreased formation of white and beige adipocytes. These changes can disturb systemic metabolic homeostasis. This figure was created using Servier Medical Art (http://smart.servier.com/).

adipose-specific *Mdm2*-knockout mice [58▪] or muscle-specific mitochondrial fusion protein optic atrophy 1 (*Opa1*) deficient mice [67]. Induction of senescence in isolated primary hepatocytes promoted steatosis due to mitochondrial dysfunction followed by reduced fatty acid oxidation capacity [33▪▪]. One of the pathways involved in the development of age-associated hepatic steatosis might be the Cdk4-C/EBPα-p300 axis, as inhibition of cyclin dependent kinase 4 (Cdk4) reversed hepatic steatosis via reduction in C/EBPα-p300 complexes, resulting in reduction of senescent cells and alterations of chromatin structures in hepatocytes [68]. Importantly, senescent cells seem to contribute to NAFLD, as elimination of senescent cells, using the *INK-ATTAC* transgene reduced fat accumulation in the liver of aged, obese and diabetic mice [33▪▪] (Table 1) (Fig. 2).

A key factor in the transition of NAFLD to NASH is the activation of innate immune cells, which initiates and amplifies hepatic inflammation. Senescent cells in the liver can promote inflammation by secretion of SASP factors. The innate immune-sensing mechanism cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING) are important SASP regulators [69▪–72▪]. Loss of the cGAS-STING pathway in senescent cells greatly compromises SASP factor secretion [69▪,71▪,72▪]. Consistent with this, NAFLD patients show increased expression of STING in nonparenchymal liver cells [73▪]. Interestingly, STING activation induces liver steatosis and inflammation [74▪] and STING deficiency attenuated steatosis, fibrosis and inflammation in murine models of NASH [73▪,74▪]. It should be noted that in those studies, the effects were ascribed to activated macrophages and Kupffer cells, while senescence was not addressed. Future studies are required to reveal whether the interplay between STING activity, SASP and hepatic lipid accumulation accelerates NAFLD development and progression.

Together, these studies suggest that senescent cells in the liver may contribute to the progression of NAFLD. However, senescence might also have beneficial roles in NAFLD under certain circumstances. In a toxin-induced liver damage model, senescence of activated stellate cells limited the progression of fibrosis [75]. In addition, the role of senescent cells and cGAS-STING in the development of HCC may be context-dependent. For example, in a mouse model of HCC with persistent overexpression of oncogenic Ras, deficiency of STING resulted in intrahepatic tumour formation due to loss of immune-mediated clearance of premalignant hepatocytes [69▪]. However, HCC development was diminished in the same *Sting* knockout mice under conditions of a single dose of carcinogen treatment followed by 30 weeks of high fat diet [70▪]. Therefore, it is

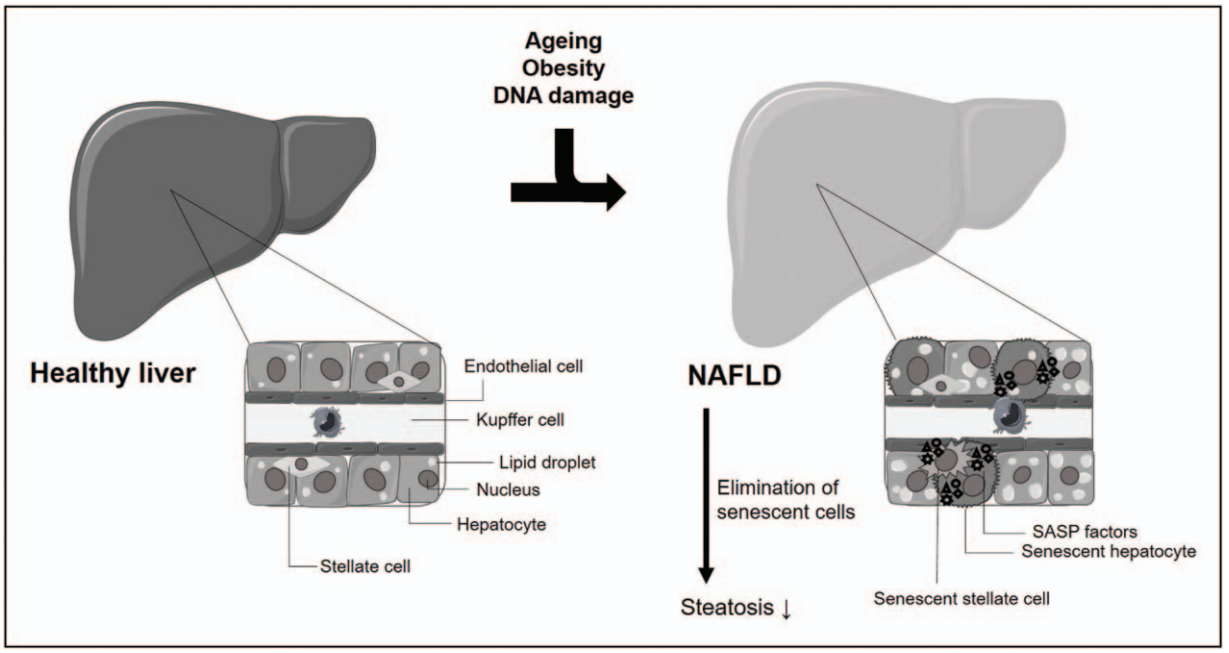

FIGURE 2. Potential role of senescent cells in the development of hepatic steatosis. Senescent hepatocytes induce lipid accumulation. Senescent stellate cells secrete SASP factors, which can trigger activation of immune cells such as Kupffer cells leading to NAFLD progression. This figure was created using Servier Medical Art (http://smart.servier.com/).

possible that cGAS-STING activation and SASP from acutely generated senescent cells promote immune-surveillance, whereas long-term exposure to SASP factors of obesity-induced senescent hepatic stellate cells acts detrimental.

Role of cellular senescence in atherosclerosis

Ageing is accompanied by proatherogenic changes in the vasculature, including arterial stiffness, calcification and increased arterial permeability [76]. The presence of metabolic derangements, such as dyslipidaemia, initiates and accelerates atherosclerotic plaque formation. Interestingly, genome-wide association studies (GWAS) revealed that polymorphisms at the chromosome 9p21 locus are the most robust genetic markers for atherogenesis [77]. These associations were independent of established cardiovascular risk factors, such as blood lipid levels. Although studies have suggested that this locus owes its functional relevance to the long coding RNA *ANRIL* (antisense noncoding RNA in the INK4 locus) [78,79], it also encodes the cyclin-dependent kinase inhibitors and major regulators of senescence $p16^{INK4A}$, $p15^{INK4B}$ and the p53 regulatory protein $p14^{ARF}$. Vascular smooth muscle cells (VSMCs) and vascular endothelial cells derived from human atherosclerotic plaques display features of senescence, including SA-β-gal activity, increased expression of $p16^{INK4A}$ and *p21* and hypophosphorylation of the retinoblastoma tumour suppressor protein Rb [80–83]. However, it remains unclear whether cellular senescence also contributes to atherosclerosis development. Mice deficient in $p19^{Arf}$, p21 and p53 display accelerated atherosclerosis development and, although these cell cycle regulators are involved in many processes and findings of these studies were mainly attributed to effects on apoptosis [84–86], a role of cellular senescence in atherogenesis cannot be excluded. Whether cellular senescence of VSMCs is beneficial or adverse for plaque development is controversial. Gizard *et al.* [87] previously demonstrated protective effects of cellular senescence due to limiting proliferation and accumulation of VSMCs in the tunica intima. However, VSMC proliferation is protective in early and advanced atherosclerosis [88]. Wang *et al.* [89] reported that VSMC senescence promoted atherosclerosis development and features of plaque vulnerability. Mechanistically, senescent cells might promote plaque formation and vulnerability via pro-inflammatory SASP cytokines that could facilitate macrophage influx as well as matrix-degrading SASPs that could trigger plaque rupture [81,90]. The only conclusive evidence for a role of cellular senescence in atherosclerosis comes from a study by Childs *et al.* [91▪] that used both pharmacological and INK-ATTAC mediated clearance of senescent cells in atherosclerosis prone *LDL receptor* knockout

mice and convincingly demonstrated that removal of $p16^{Ink4a}$-positive foamy macrophages blocks lesion growth and promotes plaque remodelling associated with plaque stability. Overall, these findings demonstrate that the role of VSMC senescence in atherosclerotic plaque development is inconclusive, whereas accumulation of $p16^{Ink4a}$-positive foamy macrophages might be detrimental for lesion progression and stability.

CONCLUSION

Cellular senescence has a causal role in adipose tissue dysfunction, presumably through induction of low-grade inflammation and inhibition of adipogenic differentiation resulting in insulin resistance, dyslipidaemia and ultimately development of cardiometabolic disease. Removal of senescent cells can potentially play an important role in treatment or prevention of these diseases. Current senolytic agents interfere with the pro-survival pathways, on which senescent cell survival depends. An alternative approach might be to target the secretion or activity of SASP factors, as these are likely to facilitate systemic tissue dysfunction.

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Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
- ■■ of outstanding interest

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